Flow Cytometric Analysis of CD4 and CD8 T Cells in Spleen of Chicken (Gallus domesticus)

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ABSTRACT

Flow cytometric analysis of CD4 and CD8 T cells of spleen was done in layer chicken of various age groups ranging from day-old to forty weeks. The mean CD4 and CD8 population was found to be the highest at day one and lowest at twenty weeks of age. However, an increase in both the T cell population was detected at forty weeks. Among the two cell types studied, CD8 cells were always higher than the CD4 cells with significant difference (P<0.05) at day one. A distinct difference was observed in the ratio of CD4 and CD8 cells in the spleen at different age.

Key words: CD4, CD8 T Cells, Spleen, Chicken

The immunocompetence of an individual is evaluated depending on several parameters such as circulating T lymphocyte populations such as CD3+, CD4+, CD8+, TCR+, TCR2+ and TCR3+ lymphocytes. The amount and proportion of T cell subsets in circulation and in organs have been correlated with disease susceptibility (Kitagawa et al., 1998). The macrophages modulate the severity of the infection whereas, the lymphocytes, in particular CD4+ T cells, act as inducer of an effective immune response (Jeurissen and Veldman, 2002). Understanding age-related immunocompetence by evaluating circulating T lymphocyte population in apparently healthy commercially raised chicken is of direct relevance to developing breeding strategies as well as promoting health measures of the flock (Zekarias et al., 2002).

Immunohistochemical study of spleen in layer chicken was conducted at the Department of Veterinary Anatomy and Histology, Madras Veterinary College. Material was collected from birds of six different age groups i.e day-old, four, eight, twelve, twenty and forty weeks age. Six birds used in each age group were procured from Poultry Research Station, Nandhanam, Chennai. CD4, CD8 count in the spleen of the birds of different age groups under study was estimated by flow cytometry in the Department of Animal Biotechnology, Madras Veterinary College. Lymphocyte suspensions were prepared from spleen as per the method of Wu et al. (2000) and the cell concentration was adjusted to 1.5 x 10⁶ cells/ml in RPMI-1640 medium.

The spleen was aseptically collected in RPMI-1640 medium containing antibiotics, washed three times in medium containing antibiotic. The capsule of spleen was removed and the tissue was teased and filtered through cheese cloth. The filtrate was then centrifuged at 1500 rpm for five minutes and the supernatant was collected and centrifuged at 550 g for 15 minutes in the present work against 550 g for ten minutes as per the method of Wu et al. (2000). The cell pellet was then washed two times with FACS buffer (PBS + 3% horse serum + 0.1% sodium azide). Ten microlitre of cell suspension was mixed with 10 microlitre of one per cent trypan blue to check the live and dead cells. The cell concentration was adjusted to 1 x 10⁶ cells /ml in FACS buffer.

A single colour immunofluorescence staining procedure was followed as per Chan et al. (1988) with the following procedure. 100 microlitre of cell suspension was taken in three microcentrifuge tubes (control without monoclonal antibodies, CD4 and CD8) and the cells were pelleted by centrifugation at 800 rpm for five minutes at 4ºC. Supernatant was discarded and ten microlitre of monoclonal antibodies (1 in 100 dilution in PBS) was added in CD4 and CD8 tubes except in the control tube. The control tube was added with 10 microlitre of FACS buffer. Mouse monoclonal antibodies such as mouse anti-chicken

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CD4 FITC (Cat. No. Serotec MICA 2164F) and mouse anti-chicken CD8a FITC (Cat. No. Serotec MCA-2166F) that recognize the avian CD4 and CD8, respectively were used. Tubes were mixed gently and incubated at 4°C for 45 minutes in dark. After incubation the cells were washed with the 100 microlitre FACS buffer and pelleted by centrifugation at 800 rpm for 5 minutes and the pelleted cells were then resuspended in 500 microlitre of FACS buffer. The cells were kept in ice until it was read in the FACS caliber flow cytometer (Becton and Dickinson, USA). The lymphocytes were gated based on the FSC and SSC characteristics and using the PBS control cells the background florescence was set as M1 and the fluorescence was set as M2. Percentage of M2 values for different monoclonal antibodies was recorded from the single parameter histogram. The ratio of CD4 and CD8 was calculated by dividing percentage of CD4+CD8- cells by percentage of CD4- CD8+ cells.

Statistical analysis was carried out using the SYSTAT statistical analysis software (Systat Inc., Evanston, IL). Test results were considered significant of P<0.01. All data were expressed as the mean ± S.E.

In day-old birds, the mean of CD4:CD8 ratio was 0.61 in spleen and later increased to 1.01 in the spleen of four week-old birds (Fig. 1). The mean CD4 and CD8 population was found to be the highest at day one and lowest at twenty weeks of age. An increase in both the T cell population was detected at forty weeks of age. A reverse condition was recorded by Erf (1997) in commercial broilers and specific pathogen single comb white leghorn birds.

In eight weeks, the mean CD4:CD8 in spleen was 0.95. Bridle et al. (2006) detected an increase in the circulating CD4 and CD8 T cell populations by eight weeks. Although we have not assessed the circulating T cell population in our study, the increase in the population of these cells in spleen at two hundred days concurred with the findings of Bridle et al. (2006). Among the two cell types studied, CD8 cells were always higher than the CD4 cells, with significant difference (P<0.05) at day one (Fig. 1).

The mean of the CD4:CD8 was found to be 0.62 and 0.43 in spleen of twelve and twenty week-old birds, respectively. Whereas, in forty weeks of age, it was recorded as 0.75. Erf et al. (1998) recorded decreased CD4:CD8 between two and seven weeks of age in commercial broiler chicken. The CD4 and CD8 ratio in spleen was less at day one and high at eight weeks. However, the ratios narrowed down at forty weeks of age (Fig. 1).

REFERENCES


![Fig. 1. Mean CD4 and CD8 T cells in spleen of chicken of different age groups.](image-url)